RESEARCH ARTICLE
Bull Biotechnol (2021) 2(2):23-26
https://doi.org/10.51539/biotech.1027492 Jurnal homepage: www.dergipark.org.tr/biotech

Bulletin of Biotechnology

Tomentosin shows anticancer effect on U87 human glioblastoma multiforme cells

İlknur Çınar Ayan[1], Ebru Güçlü[1*], Hatice Gül Dursun[1], Hasibe Vural[1]

[*1]*Department of Medical Biology, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Türkiye*

*Corresponding author : ebruavc.87@gmail.com
Orcid No: https://orcid.org/0000-0001-5330-6159

Received : 23/11/2021
Accepted : 21/12/2021

Abstract: Glioblastoma multiforme (GBM) is one of the most common brain tumors. Chemotherapy, radiotherapy and surgical resection are methods used in GBM treatment, however, the investigation of possible anticancer effects of low-toxicity natural products on various cancer cells, including GBM, leads to promising results. In this study, it was aimed to investigate anticancer effect of tomentosin, which is a sesquiterpene lactone, on U87 human GBM cells. The cytotoxic effect of tomentosin was evaluated by XTT assay. The concentration of tomentosin that inhibits 50% cell viability (IC_{50}) was determined by the results from XTT, and, in further analyzes cells were treated with tomentosin at IC_{50} concentration. Then, total RNA isolation and cDNA synthesis were performed in control and dose groups, and, the possible anticancer effect of tomentosin was determined by evaluating the expression levels of important genes associated with apoptosis and metastasis by qPCR analysis. In addition, the effect of tomentosin on the colony forming capacity of GBM cells was evaluated by colony formation assay. According to our results, IC_{50} dose of tomentosin was found to be 28.8 µM in U87 cells for 48 hours. When compared to the control group, tomentosin increased expression of *BAX*, *CASP3*, *CASP8*, *CASP9*, *CYCS*, *FADD*, *TNF*, *TNFR1*, *TNFR2* and *TIMP2* genes. And, tomantosin significantly decreased colony forming capacity of U87 cells. In conclusion, it is thought that tomentosin exerts its anticancer effect by changing the expression levels of genes associated with apoptosis and metastasis in U87 GBM cells.

Keywords: Apoptosis; Glioblastoma multiforme; Metastasis; Tomentosin.

1 Introduction

Glioblastoma multiforme (GBM), which accounts for approximately 48% of all malignant primary brain tumors, is one of the deadliest malignancies with a 5-year survival rate of 5.5% and a median survival rate of 15 months (Choi et al. 2020, Tan et al. 2020). Standard treatment for GBM includes a combination of surgical resection, radiotherapy and temozolomide. However, both drug resistance mechanisms and GBM microenvironment limit the effectiveness of treatment methods (Bagherian et al. 2020, Cha et al. 2020).

In the last two decades, studies with cell culture and animal experiments have revealed that natural products with low toxicity can have an anticancer effect by influencing the development and progression of cancer (Kashyap et al. 2021). The identification of natural products that can cross blood-brain barrier, target oncogenic signaling pathways and increase sensitivity to chemotherapy and radiotherapy is very important for the development of new therapeutic strategies against GBM (Park et al. 2017, Vengoji et al. 2018).

Tomentosin, a natural sesquiterpene lactone with various biological activities such as anti-inflammatory, antibacterial and antifungal, is found in medically important members of Asteraceae family such as *Inula viscosa* (Cafarchia et al. 2001, Park et al. 2014, El Omari et al. 2021). In addition, it has been shown that tomentosin inhibits cell proliferation and has an anticancer effect in various cancers such as hepatocellular carcinoma, osteosarcoma, leukemia and gastric cancer (Lee et al. 2019, Yang et al. 2020, Yang et al. 2021, Yu et al. 2021). In this study, it was aimed to investigate the possible anticancer effect of tomentosin on U87 human GBM cells. For this, while determining the cytotoxic and antiproliferative effects of tomentosin were determined on GBM cells, its effect on expression levels of important genes in apoptosis and metastasis was also evaluated. In addition, the effect of tomentosin on the colony forming capacity of U87 cells was investigated.

2 Materials and Method

2.1 Cell culture

U87 (ATCC® HTB-14™) human GBM cell line was purchased from American Type Culture Collection (ATCC, USA). The cells were cultured in high-glucose DMEM medium containing 10% fetal bovine serum and 1% penicillin–streptomycin at 37 ℃, 95% humidity and 5% CO_2.

2.2 Cytotoxicity assay

The cytotoxic effect of tomentosin on U87 cells was determined by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) analysis. For this, U87 cells (10^4 cells/well) were seeded into 96-well plates. After 24 hours, cells were treated with tomentosin at concentrations of 0, 5, 10, 15, 20, 25, 30, 40, 50, 75 and 100 µM for 24, 48 and 72 hours. At the end of the incubation period, XTT solution (Biological Industries, 20-300-1000) was added to each well and absorbance values were read in a microplate reader (BioTek Epoch) at 450 nm wavelength and 630 nm reference range after 4 hours. The concentration of tomentosin that inhibits 50% cell viability (IC_{50}) was calculated by GraphPad Prism 8.0.2 software using XTT data and in further analyzes cells were treated with tomentosin at IC_{50} concentration.

2.3 RNA isolation, cDNA synthesis and qPCR analysis

In this study, expression levels of *BAX*, *BCL2*, *CASP3*, *CASP7*, *CASP8*, *CASP9*, *CASP10*, *CYCS*, *PPARG*, *FAS*, *FADD*, *TNF*, *TNFR1*, *TNFR2*, *MMP2*, *MMP9*, *TIMP1*, *TIMP2*, *CDH1* and *CDH2* genes were evaluated with qPCR. For this, total RNA isolation was conducted with RiboEx (GeneAll, 301-001) in control and dose groups. cDNA synthesis was performed in accordance with the manufacturer's instructions (Bio-Rad, 170-8891). qPCR was performed with reaction mixes containing 5 µl qPCR MasterMix (BrightGreen 2X qPCR MasterMix – ROX, ABM, MasterMix-R), 5 pMol forward primer, 5 pMol reverse primer, and 2 µl cDNA. The reaction was carried out in a Real-time PCR System (Bio-Rad, CFX Connect) for 40 cycles by applying the protocol consisting of enzyme activation (10 minutes at 95˚C), denaturation (15 seconds at 95˚C) and annealing/extension (60 seconds at 60˚C). Analysis was performed by $2^{-\Delta\Delta CT}$ method using GAPDH reference gene.

2.4 Colony formation assay

Control and dose groups cells were seeded at a density of 2×10^3 cells/well into 6-well plate. Cells were incubated at 37°C for 10 days and medium was changed every 2 days. After incubation, cells were fixed with cold methanol for 10 min and stained with 0.5% crystal violet dye. Analysis was performed after counting the stained colonies.

2.5 Statistical analysis

Statistical analysis was performed using t-tests in GraphPad Prism version 8.0.2. "RT2 Profiler ™ PCR Array Data Analysis" program was used quantitative analysis of gene expression results. All experiments were performed in triplicate and data were expressed as mean ± standard deviation. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Tomentosin inhibits U87 cell proliferation

In order to determine the cytotoxic effect of tomentosin, U87 cells were treated with different concentrations of tomentosin for 24, 48 and 72 hours and XTT analysis was performed. According to the results, tomentosin inhibited U87 cell proliferation in a dose- and time-dependent manner (Fig 1). In addition, IC_{50} dose of tomentosin was found to be 28.8 µM in U87 cells for 48 hours. In subsequent analyses, cells were treated with 28.8 µM tomentosin for 48 hours.

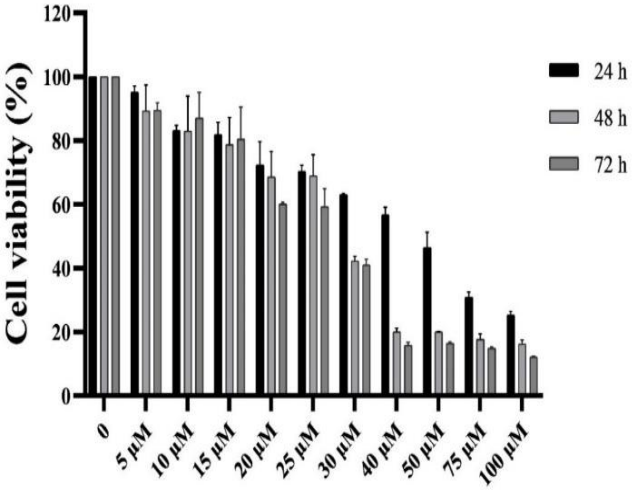

Fig. 1 Effect of tomentosin on U87 cells viability. U87 cells viability (%) was determined by XTT analysis. IC_{50} dose of tomentosin was calculated using XTT data and was found to be 28.8 µM for 48 hours.

3.2 Tomentosin effects expressions of genes associated with apoptosis and metastasis

The effect of tomentosin on expression levels of important genes associated with apoptosis and metastasis was determined by qPCR analysis. Accordingly, expression levels of *BAX*, *BCL2*, *CASP3*, *CASP7*, *CASP8*, *CASP9*, *CASP10*, *CYCS*, *PPARG*, *FAS*, *FADD*, *TNF*, *TNFR1* and *TNFR2* genes associated with apoptosis and *MMP2*, *MMP9*, *TIMP1*, *TIMP2*, *CDH1* and *CDH2* genes associated with metastasis were evaluated in qPCR. After treatment with IC_{50} dose of tomentosin, expression level of *BAX*, *CASP3*, *CASP8*, *CASP9*, *CYCS*, *FADD*, *TNF*, *TNFR1*, *TNFR2* and *TIPM2* genes significantly increased to 2.23, 2.85, 1.75, 1.70, 2.5, 1.52, 3.78, 3.84, 3.81 and 2.12 folds, respectively, compared with control group ($p<0.05$). No significant change was observed in the expression level of *BCL2*, *CASP7*, *CASP10*, *PPARG*, *FAS*, *MMP2*, *MMP9*, *TIMP1*, *CDH1* and *CDH2* genes (Fig 2).

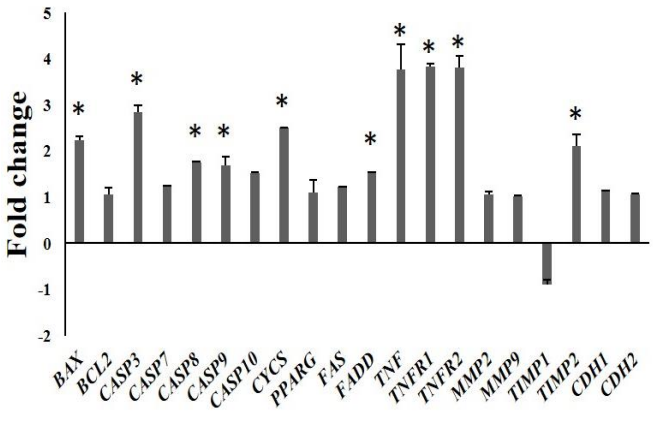

Fig. 2 Effect of tomentosin on expression of important genes in apoptosis and metastasis. *P < 0.05.

3.3 Tomentosin inhibits colony formation of U87 cells

Colony formation assay was performed to determine the effect of tomentosin on growth of U87 cells. Accordingly, tomentosin significantly reduced colony formation of U87 cells (Fig 3).

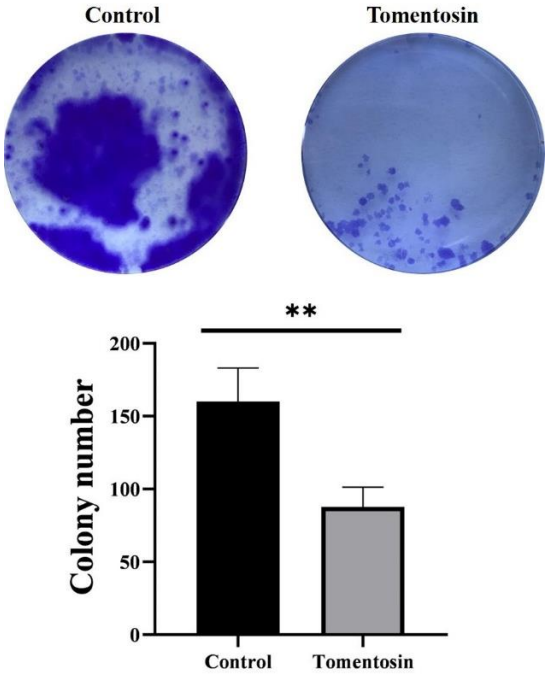

Fig. 3 Effect of tomentosin on colony formation of U87 cells. Cells were treated with IC_{50} dose of tomentosin for 48 hours and cultured for 10 days. **P < 0.01.

4 Discussion

Tomentosin, whose possible anticancer effect on GBM cells was investigated in this study, is a natural sesquiterpene lactone with important biological activities. Sesquiterpene lactones are known to be found in medicinal plants, and it is stated that they cause anticancer effects by changing the redox cell balance and by affecting NF-κB and STAT3, in addition to their various pharmacological properties (Babaei et al. 2018). Studies with various cancer cell lines have also reported the anticancer effect of tomentosin as a sesquiterpene lactone (Merghoub et al. 2017, Lee et al. 2019, Yang et al. 2020, Virdis et al. 2021, Yang et al. 2021, Yu et al. 2021). Virdis et al. (2021) has been shown that tomentosin has an anticancer effect on Burkitt's lymphoma (BL) cells by arresting cell cycle and inducing apoptosis. In addition, researchers stated that tomentosin decreases the expression of anti-apoptotic genes such as *BCL2A1* and *CDKN1A*, increases the expression of *PMAIP1* proapoptotic gene. Similarly, in a study with hepatocellular carcinoma cells, it has been shown that tomentosin induces apoptosis and arrests cell cycle (Yu et al. 2021). And also, it has been stated that tomentosin induces the mitochondria-mediated apoptotic pathway through an increase in the level of reactive oxygen species in leukemia, osteosarcoma, cervical cancer and gastric cancer cells (Merghoub et al. 2017, Lee et al. 2019, Yang et al. 2020, Yang et al. 2021).

In this study, tomentosin inhibited proliferation of U87 human GBM cells. It also increased the expression of genes encoding important proteins involved in the intrinsic and extrinsic pathways of apoptosis (Fulda and Debatin 2006). And, it caused an increase in the expression of *TIMP2*, is effective in the inhibition of MMP2 which provides invasion of cancer cells by destructing of extracellular matrix (Arpino et al. 2015). According to the results of the colony formation analysis, tomenstosin inhibited the U87cell growth and decreased the colony forming capacity of the cells.

5 Conclusion

As a result, it is thought that tomentosin may cause anticancer effects by changing the expression levels of important genes known to play a role in apoptosis and metastasis in U87 human GBM cells. However, further analyzes are required to fully elucidate the anticancer mechanism of tomentosin in GBM.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions: ICA:Investigation, methodology, formal analysis, writing-original draft, writing-review& editing, visualization. EG: Investigation, methodology,formal analysis, writing-original draft, writing-review&editing, visualization. HGD: Investigation, writing-review&editing, supervision. HV: Investigation, writing-review&editing, supervision.

Conflict of interest disclosure: The authors declare that they have no conflict of interest.

References

Arpino V, Brock M, Gill SE (2015) The role of TIMPs in regulation of extracellular matrix proteolysis. Matrix Biol 44-46:247-54.

Babaei G, Aliarab A, Abroon S, Rasmi Y, Aziz SG (2018) Application of sesquiterpene lactone: A new promising way for cancer therapy based on anticancer activity. Biomed Pharmacother 106:239-246.

Bagherian A, Mardani R, Roudi B, Taghizadeh M, Banfshe HR, Ghaderi A, Davoodvandi A, Shamollaghamsari S, Hamblin MR, Mirzaei H (2020) Combination Therapy with Nanomicellar-Curcumin and Temozolomide for In Vitro Therapy of

Glioblastoma Multiforme via Wnt Signaling Pathways. J Mol Neurosci 70(10):1471-1483.

Cafarchia C, De Laurentis N, Milillo MA, Losacco V, Puccini V (2001) Fungistatic activity of a sesquiterpene lactone (tomentosin) isolated from fresh Inula viscosa (Asteraceae) flowers from the Puglia region. Parassitologia 43(3):117-21

Cha GD, Kang T, Baik S, Kim D, Choi SH, Hyeon T, Kim DH (2020) Advances in drug delivery technology for the treatment of glioblastoma multiforme. J Control Release 328:350-367.

Choi J, Kim G, Cho SB, Hyung-Jun I (2020) Radiosensitizing high-Z metal nanoparticles for enhanced radiotherapy of glioblastoma multiforme. J Nanobiotechnol 18, 122.

El Omari N, El Menyiy N, Zengin G, Goh BH, Gallo M, Montesano D, Naviglio D, Bouyahya A (2021) Anticancer and Anti-Inflammatory Effects of Tomentosin: Cellular and Molecular Mechanisms. Separations 8, 207.

Fulda S, Debatin KM (2006) Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene 25, 4798–4811.

Kashyap D, Tuli HS, Yerer MB, Sharma A, Sak K, Srivastava S, Pandey A, Garg VK, Sethi G, Bishayee A (2021) Natural product-based nanoformulations for cancer therapy: Opportunities and challenges. Semin Cancer Biol 69:5-23.

Lee CM, Lee J, Nam MJ, Choi YS, Park SH (2019) Tomentosin Displays Anti-Carcinogenic Effect in Human Osteosarcoma MG-63 Cells via the Induction of Intracellular Reactive Oxygen Species. Int J Mol Sci 20(6):1508.

Merghoub N, El Btaouri H, Benbacer L, Gmouh S, Trentesaux C, Brassart B, Attaleb M, Madoulet C, Wenner T, Amzazi S, Morjani H, El Mzibri M (2017) Tomentosin Induces Telomere Shortening and Caspase-Dependant Apoptosis in Cervical Cancer Cells. J Cell Biochem 118(7):1689-1698.

Park HH, Kim SG, Kim MJ, Lee J, Choi BK, Jin MH, Lee E (2014) Suppressive effect of tomentosin on the production of inflammatory mediators in RAW264.7 cells. Biol Pharm Bull 37(7):1177-83.

Park MN, Song HS, Kim M, Lee MJ, Cho W, Lee HJ, Hwang CH, Kim S, Hwang Y, Kang B, Kim B (2017) Review of Natural Product-Derived Compounds as Potent Antiglioblastoma Drugs. Biomed Res Int 2017:8139848.

Tan AC, Ashley DM, López GY, Malinzak M, Friedman HS, Khasraw M (2020) Management of glioblastoma: State of the art and future directions. CA Cancer J Clin 70(4):299-312.

Vengoji R, Macha MA, Batra SK, Shonka NA (2018) Natural products: a hope for glioblastoma patients. Oncotarget 9: 22194-22219.

Virdis P, Marchesi I, Fiorentino FP, Migheli R, Sanna L, Bordoni V, Pintore G, Galleri G, Muroni MR, Bagella L, Fozza C, De Miglio MR, Podda L (2021) Tomentosin a Sesquiterpene Lactone Induces Antiproliferative and Proapoptotic Effects in Human Burkitt Lymphoma by Deregulation of Anti- and Pro-Apoptotic Genes. Life 11(11):1128.

Yang H, Zhao H, Dong X, Yang Z, Chang W (2020) Tomentosin induces apoptotic pathway by blocking inflammatory mediators via modulation of cell proteins in AGS gastric cancer cell line. J Biochem Mol Toxicol 34(8):e22501.

Yang L, Xie J, Almoallim HS, Alharbi SA, Chen Y (2021) Tomentosin inhibits cell proliferation and induces apoptosis in MOLT-4 leukemia cancer cells through the inhibition of mTOR/PI3K/Akt signaling pathway. J Biochem Mol Toxicol 35(4):e22719.

Yu SH, Lee CM, Ha SH, Lee J, Jang KY, Park SH (2021) Induction of cell cycle arrest and apoptosis by tomentosin in hepatocellular carcinoma HepG2 and Huh7 cells. Hum Exp Toxicol 40(2):231-244.